

REPAYMENT OF THE ANAEROBIC OXYGEN DEBT IN GRASSHOPPER SKELETAL MUSCLE

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It has been shown (Gilmour, 1940) that in the roach *Cryptocercus punctulatus* Scudder, the "oxygen debt" incurred during a period of anaerobiosis is repaid threefold. Since such a large excess oxygen consumption during recovery had not previously been demonstrated in animal tissues, it seemed that further work on the phenomenon of oxygen debt repayment in insects was warranted.

In order to simplify the problem, it was decided to limit this investigation, as far as was possible, to one tissue. To this end, the hind femora of grasshoppers were employed as material. The femur of the jumping leg of the grasshopper consists almost entirely of skeletal muscle; the amount of epidermal and other tissue present would account for only a very small fraction of the total respiration.

MATERIAL AND METHODS

Two species of grasshopper were used: (1) *Melanoplus femur-rubrum* (De Geer). Adults of this species were collected in the field during the fall of 1939 and kept in the laboratory until used. (2) *Melanoplus differentialis* (Thomas). A number of adults were raised from eggs supplied by Dr. J. H. Bodine from the stocks at Iowa State University.

The legs were removed from the bodies by cutting through the trochanter. The tibiae were cut off close to the proximal end.

Oxygen uptake was studied in a differential volumeter designed especially for following oxygen debt repayment, the "apparatus B" previously employed by Rotta and Stannard (1939). The electrical conductivity method of Fenn (1928) was used in following carbon dioxide production. Oxygen uptake and carbon dioxide production of the resting leg were first followed in air, after which the vessels were filled with nitrogen, by running through the pure gas for 20 minutes,

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and the carbon dioxide production during anaerobiosis was determined; finally air or oxygen was re-admitted and the recovery respiration followed until a steady rate of oxygen uptake had been reestablished.

It was not possible to use the respirometer designed for conductivity determination for following both oxygen uptake and carbon dioxide production during the recovery period, as the oxygen uptake readings were disturbed by solubility effects. The anaerobic period was ended by passing pure oxygen through the vessel for one minute, insufficient time for equilibrium to be established between the gas mixture and the relatively large volume of barium hydroxide required by the method. Oxygen uptake was followed, however, at the beginning and end of

TABLE I

Repayment of oxygen debt in isolated hind femora of *Melanoplus femur-rubrum*. The duration of anaerobiosis sometimes varied slightly from that shown in column 2. Such variations have been considered in calculating the amount of oxygen missed.

Experiment	Time in N ₂	Initial O ₂ uptake	O ₂ missed	Final O ₂ uptake	Excess O ₂ consumed	$\frac{\text{Excess O}_2 \text{ consumed}}{\text{O}_2 \text{ missed}} \times 100$
	minutes	mm. ³ /gm./hr.	mm. ³ /gm.	mm. ³ /gm./hr.	mm. ³ /gm.	
G3	30	218	110	180	193	175
G5	30	180	90	173	160	180
G6	30	186	99	179	160	160
G9	30	269	135	277	250	185
G12	30	191	96	180	262	275
G4	60	158	158	162	173	110
G8	60	140	140	166	196	140
G10	60	201	201	162	550	275
Average		193		185		190

each run with the conductivity apparatus, in order to establish the respiratory quotient.

The temperature of the experiments was 23° C.

That an adequate supply of oxygen to the interior of the excised legs was maintained by diffusion from air was demonstrated by the fact that filling the vessels with oxygen caused no increase in oxygen consumption above that measured in air. The legs survived without any apparent disturbance in respiratory metabolism throughout the course of the experiments (5 to 9 hours). The oxygen uptake usually remained constant over long periods of time, although the final steady rate was often slightly lower than the initial.

EXPERIMENTAL RESULTS

M. femur-rubrum

Either two or three legs were used in each experiment. The average oxygen uptake was 180 cu.mm. per gram live weight per hour (26 determinations ranging between 140 and 277 cu.mm. per gram per hour). The results of the oxygen debt experiments are shown in Table I. "Oxygen missed," in this table, means the amount of oxygen the tissue would have consumed in air, during the time it was in nitrogen. The last column represents the percentage repayment of the oxygen debt.

TABLE II

Repayment of oxygen debt and retention of carbon dioxide in isolated hind femora of *Melanoplus differentialis*.

Experiment	Initial O ₂ uptake	RQ	Final O ₂ uptake	RQ	Percentage repayment of O ₂ debt	CO ₂ produced in N ₂	Expected excess CO ₂ production	Measured excess CO ₂ production	CO ₂ retained
	<i>mm.³/gm./hr.</i>		<i>mm.³/gm./hr.</i>			<i>mm.³/gm.</i>	<i>mm.³/gm.</i>	<i>mm.³/gm.</i>	<i>mm.³/gm.</i>
M1	178	—	164	—	120	—	—	—	—
M2	180	0.93	180	0.80	—	136	225	212	13
M5	132	—	134	—	175	—	—	—	—
M6	142	0.79	132	0.85	—	126	178	174	4
M7	198	—	186	—	115	—	—	—	—
M8	212	0.88	216	0.79	—	154	265	20	245
M10	205	0.94	183	0.85	—	116	256	88	168
M13	207	—	195	—	160	—	—	—	—
M14	243	0.67	234	0.67	—	100	304	166	138
M15	253	—	212	—	80	—	—	—	—
M16	277	0.72	237	0.73	—	87	346	194	152
M17	202	—	201	—	110	—	—	—	—
M18	187	0.98	207	0.89	—	123	234	176	58
Average	201	0.84	191	0.80	125	120	—	—	111

There is no significant difference between the value for this obtained from the half-hour experiments and that from the one-hour experiments.

M. differentialis

One leg was used in each experiment. The procedure was to use the femur of one side of the grasshopper in the oxygen debt apparatus, and that of the other side in the respirometer designed for conductivity determination.

The average oxygen uptake was 197 cu.mm. per gram per hour (29 determinations ranging between 132 and 277 cu.mm. per gram per hour). Table II shows the results for both oxygen uptake and carbon dioxide production. The percentage repayment of the oxygen debt is calculated in the same manner as in Table I. The duration of anaerobio-

sis was one hour in all experiments. The "expected excess carbon dioxide production" is the amount of carbon dioxide which would have been given off, over and above that produced as the result of basal metabolism, if the recovery process had had a respiratory quotient of 1.0, and there had been no retention. The carbon dioxide retained in the tissues during recovery is the difference between this figure and the measured excess carbon dioxide production. The figures obtained in this way show an extremely wide range of variation, but have an average which is approximately equal to the average amount of carbon dioxide produced during anaerobiosis. The variation must be due largely to the fact that oxygen uptake and carbon dioxide production during recovery were not determined on the same tissue. In determining the expected excess carbon dioxide production it was supposed that the repayment of oxygen debt had the average value (125 per cent) in each case; that is, that the figure for excess oxygen consumed (and hence for carbon dioxide produced) was 125 per cent of the original oxygen uptake per hour. It might be supposed that a more accurate method would be to use, in each of the carbon dioxide experiments, the figure for oxygen debt repayment obtained from the opposite leg of the same grasshopper. When this is done, however, the variation is as great, while the average is practically unchanged (106 cu.mm. per gram). It appears, then, that it is impossible to predict the actual percentage repayment of oxygen debt of any leg, even from an experiment run on another leg of the same grasshopper. The average is thus the only figure for carbon dioxide retention that need be considered.

The respiratory quotient is somewhat low for muscle, but at the time at which experiments M14 and M16 were run the insects were rather inadequately fed, and the low respiratory quotients in these experiments are probably the result of the utilization of reserve foodstuffs.

DISCUSSION

Since the chemical constituents of insect muscle are quite similar to those of vertebrate muscle, it is not unreasonable to expect the anaerobic processes of the two groups to be at least qualitatively similar. The fact that in *M. differentialis* the amount of carbon dioxide produced during anaerobiosis is equal to the amount retained during recovery suggests that the amount produced in anaerobiosis is the result simply of the buffering of acid by bicarbonate, and supports the conclusion that lactic acid is the only important end-product of anaerobiosis. In frog muscle 70 per cent of the debt incurred during anaerobiosis is repaid (Rotta and Stannard, 1939). In the insects used in this study more than 100

per cent is repaid. The problem of the removal of lactic acid thus seems to be a more expensive one in grasshopper muscle than in frog muscle, particularly in the case of *M. femur-rubrum*, which uses more oxygen in the recovery process than does *M. differentialis*. It has already been suggested that in *Cryptocercus* (*loc. cit.*) the threefold repayment of the oxygen debt may have been due to the burning off of a large proportion of the lactic acid supposed to have been produced by anaerobiosis. The fact that in isolated muscle tissue, investigated at normal temperature (as *Cryptocercus* was not), a repayment of the oxygen debt in excess of 100 per cent can be demonstrated lends support to this conclusion.

SUMMARY

The average oxygen consumption of isolated hind femora of *Melanoplus femur-rubrum* was 180 cu. mm. per gram per hour; that of femora of *M. differentialis* was 197 cu. mm. per gram per hour. The average respiratory quotient of the latter was 0.82.

In *M. femur-rubrum* 190 per cent of the oxygen debt incurred during anaerobiosis was repaid during recovery. In *M. differentialis* 125 per cent of the debt was repaid, and the carbon dioxide retained in the tissues during the recovery period was equal to the carbon dioxide produced during anaerobiosis.

The end-products of anaerobiosis in grasshoppers are probably similar to those in vertebrates, but their removal seems to involve a greater expenditure of energy.

My thanks are due to Dr. W. O. Fenn for making available the facilities of his laboratory, and for his interest in the course of this work.

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